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Sodium Chloride, Potassium Chloride, and Virulence in *Listeria monocytogenes*

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Virulence, as determined in a mouse model, and the virulence factor activities of catalase, superoxide dismutase, and listeriolysin O were examined in a parental strain (10403S) and in a nonhemolytic mutant strain (DP-L224) of *Listeria monocytogenes*. The cells were propagated in media containing various concentrations of sodium chloride or potassium chloride. Strains 10403S and DP-L224 exhibited significant increases in catalase activity and listeriolysin O activity when grown in medium containing either salt at 428 mM. The superoxide dismutase activities for both strains increased when they were grown in medium containing either salt. The superoxide dismutase activity was significantly increased only when cells were propagated in medium containing no salt compared with that when they were propagated in medium containing either salt at 1,112 mM. In addition, the listeriolysin O activity was highest for cells propagated in medium containing KCl at 428 mM, while the activity was significantly less for cells propagated in medium containing NaCl at an equal concentration. Virulence was examined in mouse livers and spleens after intravenous infection, and approximate 50% lethal doses were determined after intragastric and intraperitoneal infection. Each method of infection indicated that listeriolysin O is required for virulence, while growth in salt-containing medium or the production of higher levels of catalase, superoxide dismutase, and listeriolysin O do not appear to enhance the virulence of *L. monocytogenes*.

Listeria monocytogenes is an ubiquitous bacterium, is gram positive, grows aerobically or facultatively anaerobically, and is an intracellular pathogen. It is a salt-tolerant organism that is capable of growing at temperatures ranging from 1 to 44°C (18). Seeliger and Welshimer (26) demonstrated that *L. monocytogenes* could survive in 20% NaCl for 8 weeks at 4°C, while Shahamat et al. (27) reported that the organism could survive for 15 days in medium containing 10.5% NaCl at 37°C. McClure et al. (20) showed that *L. monocytogenes* grows in medium containing 10% NaCl at pH 7.0 and 25°C.

The organism is considered a major foodborne pathogen which is found in a variety of foods. It generally causes disease in elderly and immunocompromised individuals, pregnant women, and newborns. Bacterial meningitis is the most common form of listeriosis. The virulence factors of *L. monocytogenes* have recently been reviewed (23). These factors include listeriolysin O (LLO) (3, 15), a phosphatidylinositol-specific phospholipase C, and a lecithinase. Portnoy et al. (23) divide *L. monocytogenes* infections into four stages: internalization, escape from a vacuole, nucleation of actin filaments, and cell-to-cell spread. The genes involved in each of these stages have been identified. Other postulated virulence factors include catalase (CA) and superoxide dismutase (SOD). The production of CA and SOD aids in detoxifying cytotoxic oxidants, while the production of LLO aids in intracellular invasiveness and, possibly, in iron acquisition (6). Cytotoxic oxidants are produced during the respiratory burst in macrophages and neutrophils and include the superoxide radical ($\cdot O_2^-$), hydrogen peroxide, and the hydroxyl radical ($\cdot OH$). It has been suggested that CA and SOD act by detoxifying these active oxygen species (30, 31). LLO is a pore-forming cytolysin similar to streptolysin O and pneumolysin (21) and is shown to be necessary for the virulence of *L. monocytogenes* (5).

There is evidence suggesting that the environmental conditions under which *L. monocytogenes* is grown influence its virulence (7) and its virulence factor activity (9). Sodium chloride and potassium chloride may be added to foods as flavor enhancers and as agents to reduce water activity. The purpose of the present study was to determine whether different levels of NaCl or KCl had any effect on the virulence factor activity of *L. monocytogenes*.

MATERIALS AND METHODS

Growth conditions. *L. monocytogenes* 10403S and DP-L224 were obtained from Daniel A. Portnoy, University of Pennsylvania, Philadelphia. Strain 10403S is β-hemolytic and was isolated from mice, while DP-L224 is a nonhemolytic mutant of 10403S derived via transposon mutagenesis (24).

The growth medium was modified tryptic soy broth (TSB; Difco, Detroit, Mich.). The formula for TSB without NaCl (TSBw) was as follows (per liter): 17 g of Bacto Tryptone (Difco), 3 g of Bacto Soytone (Difco), 2.5 g of glucose, and 2.5 g of K_2HPO_4. Sodium chloride was added to TSBw to give a final concentration of either 428 mM (TSBs 428), 787 mM (TSBs 787), or 1,112 mM (TSBs 1,112). Potassium chloride was added to TSBw to yield a final concentration of either 428 mM (TSBk 428), 787 mM (TSBk 787), or 1,112 mM (TSBk 1,112). All media were adjusted to pH 7.3 prior to autoclaving.

Frozen stock cultures were prepared by inoculating 9.9 ml of TSBw with 0.1 ml of an overnight stationary-phase inoculum grown in TSBw, vortexing, and freezing the mixture at −20°C. When working at salt concentrations of 787 mM or greater, strains 10403S and DP-L224 were grown to the early stationary phase in either TSBs 1,112 or TSBk

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1,112, and frozen stocks were prepared in TSBs 1,112 or TSBk 1,112, respectively, as described above. Frozen stocks were thawed and inoculated into 250-ml Erlenmeyer flasks containing 90 ml of growth medium. Strains 10403S and DP-L224 in TSBw were thawed and inoculated into either TSBw, TSBs 428, or TSBk 428. Ten milliliters of frozen stocks of the same strains in TSBs 1,112 or TSBk 1,112 were thawed and inoculated either into TSBs 787 or TSBs 1,112 or into TSBk 787 or TSBk 1,112, respectively. Cultures were grown at 37°C on a gyratory shaking (120 rpm) water bath to the early stationary phase, as determined by monitoring with a Klett-Summerson photoelectric colorimeter (Klett Manufacturing Co., New York, N.Y.). Determination of protein concentrations and enzyme activities (CA, SOD, and LLO) and preparation of cell extracts were conducted as described by Dallmier and Martin (9).

Virulence models. Virulence was examined in a mouse model by two methods. By the first method (method 1), we examined the ability of *L. monocytogenes* to colonize the livers and spleens of mice. Intravenous inoculation of mice was performed by the procedure of Czuprynski et al. (7), which used 4- to 5-week-old (C57BL/6 × DBA/2)F_1 male mice (method 1). By the second method (method 2), we used approximate 50% lethal dose (ALD_{50}) procedures as described by Pine et al. (22), which used white female ICR mice weighing 7 to 10 g. All mice were obtained from Harlan Sprague Dawley Inc. (Indianapolis, Ind.). Mice were housed in polypropylene "shoe-box" cages with stainless steel wire tops under microisolator caps. Food and water were provided ad libitum.

For method 1, strains 10403S and DP-L224 were grown to the early stationary phase in either TSBw, TSBs 428, or TSBk 428. Cells were separated by centrifugation (16,300 × *g*, 10 min), washed in sterile pyrogen-free Dulbecco phosphate-buffered saline (DPBS; Sigma Chemical Co., St. Louis, Mo.), and suspended in DPBS to obtain approximately 10^9 cells per ml. The final cell suspension was diluted 1/10 in DPBS to obtain approximately 10^5 cells per ml. This suspension was tempered to 37°C prior to injection. Cells were enumerated on McBrides *Listeria* agar (MLA; Difco) to confirm inoculum purity and number. All labware which contained the bacteria was depyrogenated prior to bacterial introduction. Plasticware was soaked overnight in a 1% solution of E-Toxa-Clean (Sigma) according to the instructions of the manufacturer (27a) and was rinsed with distilled water. Glassware was placed in an oven for 3 h at 350°C.

The portion of the tail which was to be injected was disinfected with ethanol, and the lateral tail vein was dilated by applying D-limonene (Sigma). Approximately 2 × 10^4 viable *L. monocytogenes* organisms (in a total volume of 0.2 ml) were injected into the tail vein. Three mice were injected per growth condition per strain. Control mice were injected with sterile DPBS and were housed in a cage containing *L. monocytogenes*-free mice. Mice were sacrificed 3 days postinoculation. The spleens and livers were aseptically removed and placed into separate glass tissue homogenizers which had previously been rinsed with sterile 50 mM potassium phosphate buffer containing 130 mM NaCl (PBS; pH 7.0). The organs were homogenized with 5 ml of sterile PBS as the diluent. This was regarded as the zero dilution. The homogenates were then serially diluted in sterile PBS, and 0.1-ml samples were plated onto MLA. Plates were incubated at 37°C for 48 h, and the colonies were counted. Results were expressed as mean ± standard error of the mean $\log_{10}$ *L. monocytogenes* per organ. Confirmatory tests were performed on the bacteria that grew on MLA. These confirmatory tests included cellobiose (positive) and mannitol (negative) fermentation, motility (positive), nitrate reduction (negative), and Voges-Proskauer (positive) procedures.

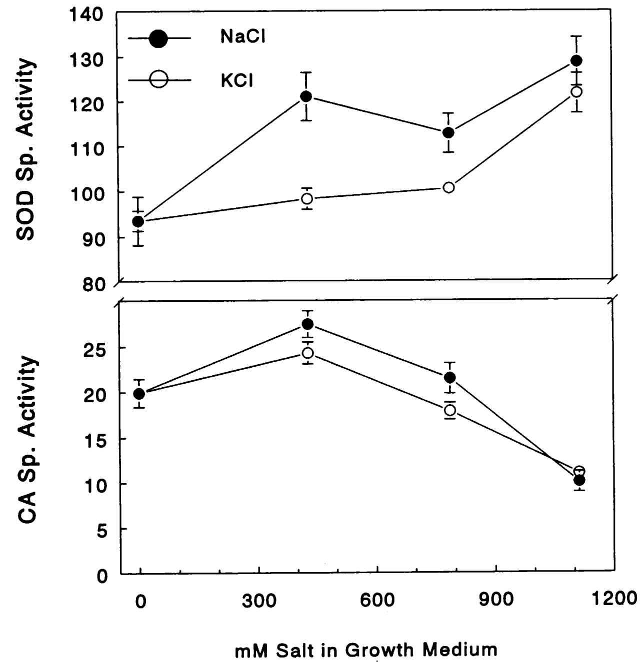

FIG. 1. Specific activities (units per milligram of protein) of the CA and SOD of *L. monocytogenes* 10403S after growth at 37°C in media containing various levels of NaCl or KCl. Error bars represent the standard errors of the means.

For method 2, strains 10403S and DP-L224 were cultured under conditions identical to those described above for method 1. Cells were centrifuged (16,300 × *g*, 10 min, 4°C) and washed twice in sterile PBS. The cells were suspended in a final volume of PBS to yield approximately 10^{11} cells per ml. Infective doses of inocula were prepared by diluting the inocula 1/10 in sterile PBS.

Mice were infected via the intraperitoneal (i.p.) or intragastric (i.g.) route. For i.p. inoculations, 0.25 ml of approximately 10^6, 10^5, 10^4, and 10^3 cells per ml was injected by using a 1-ml tuberculin syringe fitted with a 5/8-in. (1.6-cm), 25-gauge needle. For i.g. inoculations, 0.5 ml of approximately 10^{11}, 10^{10}, 10^9, and 10^8 cells per ml was injected by using a 1-ml tuberculin syringe fitted with a 1-in. (2.54-cm), 22-gauge animal feeding needle (Popper & Sons, New York, N.Y.). The moistened feeding needle was slipped past the pharynx into the stomach where the inoculum was injected. Care was taken not to injure the animals. Five mice were injected per growth condition per strain. Sterile PBS was used for the control mice. The number of viable *L. monocytogenes* organisms injected for each infective dose was verified by plating on MLA. The challenge dose for i.g. infection was chosen on the basis of a communication with L. Pine. Deaths were recorded every 24 h. ALD_{50}s, variance, and 95% confidence intervals were calculated by using a BASIC program for estimating LD_{50} values with the IBM-PC (28).

RESULTS

CA activity. The CA activities of strains 10403S and DP-L224 are shown in Fig. 1 and 2, respectively. The CA

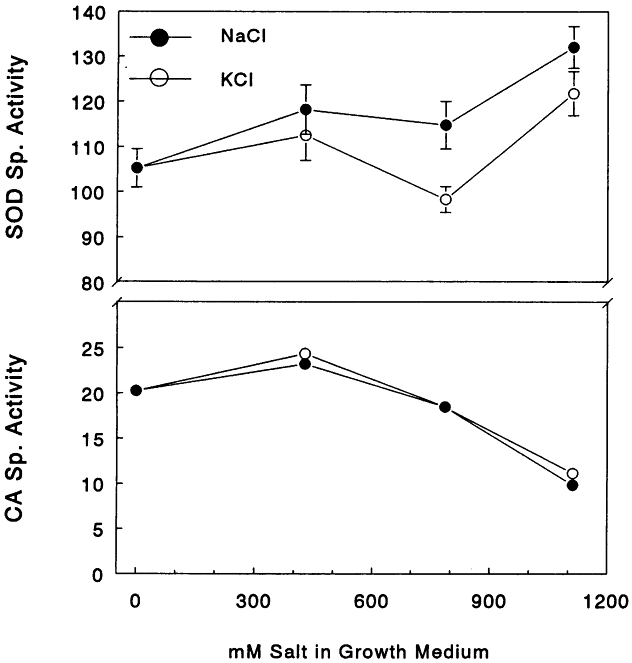

FIG. 2. Specific activities (units per milligram of protein) of the CA and SOD of *L. monocytogenes* DP-L224 after growth at 37°C in media containing various levels of NaCl or KCl. Error bars represent the standard errors of the means.

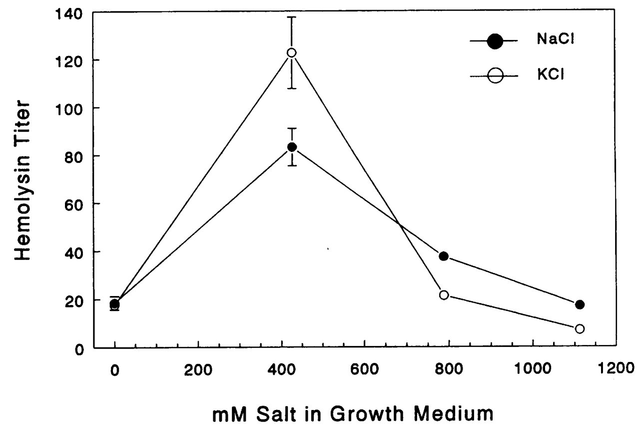

FIG. 3. Hemolysin titer (units per milligram of protein) of *L. monocytogenes* 10403S after growth at 37°C in media containing various levels of NaCl or KCl. Error bars represent the standard errors of the means.

activities of both strains were highest at 428 mM for either salt. Cells cultured in either salt at 1,112 mM exhibited approximately 50% less CA activity than cells propagated in medium containing no salt. The CA activities of each strain cultured in NaCl and KCl at equal millimolar concentrations were compared, and the activities between strains 10403S and DP-L224 cultured in equal salt concentrations were compared. None of the comparisons were significantly different ($P < 0.05$). In addition, the CA activities of each strain were compared between the salt conditions (i.e., TSBw versus TSBs 428, TSBw versus TSBs 787, TSBs 428 versus TSBs 1,112, etc.). All comparisons were significantly different ($P < 0.05$).

SOD activity. The SOD activities of strains 10403S and DP-L224 after propagation at 37°C in TSBw containing various concentrations of NaCl or KCl are shown in Fig. 1 and 2. The SOD activities of both strains increased when cells were propagated in media containing either salt at the indicated levels. Cells propagated in TSBw containing either salt at 1,112 mM exhibited the highest SOD activities. Comparisons were made between strains cultured with the same salt concentration, and comparisons were made for each strain cultured with each salt at the same millimolar concentration. In general, none of the comparisons were significantly different ($P < 0.05$). In addition, the SOD activities of each strain cultured under the different salt conditions (i.e., TSBw versus TSBs 428, TSBw versus TSBs 787, TSBs 428 versus TSBs 1,112, etc.) were compared. In general, none of the comparisons were significantly different ($P < 0.05$) except for that of cells propagated in TSBw versus cells propagated in TSBs 1,112 or TSBk 1,112.

LLO activity. The LLO titer was higher for *L. monocytogenes* 10403S cells grown in either salt at 428 mM than for cells grown in TSBw (Fig. 3). At 428 mM, KCl was more stimulatory for LLO than was NaCl. LLO titers decreased when cells were grown in either salt at concentrations greater than 428 mM. The titers of LLO dropped below the levels found in TSBw when cells were propagated in either salt at 1,112 mM. The LLO titer was not detectable for DP-L224 under any of the salt concentrations examined (data not shown).

Infection of mouse livers and spleens. The recovery of *L. monocytogenes* from the livers and spleens of mice was used as a measure of the severity of infection. Figure 4 shows the recovery of *L. monocytogenes* 10403S and DP-L224 from mouse livers and spleens. In all cases, DP-L224 was not recovered from either organ. The hemolytic strain was recovered from both mouse livers and spleens. Approximately 10 times more bacteria were recovered from the spleens than from the livers, which is similar to results reported by Leblond-Francillard et al. (16) and Czuprynski et al. (7). A two-way analysis of variance was performed on the results for *L. monocytogenes* 10403S enumerated from mouse livers and spleens. No statistically significant difference ($P < 0.05$) was seen for either organ between mice injected with bacteria propagated under the same growth conditions. In addition, no significant difference ($P < 0.05$) in

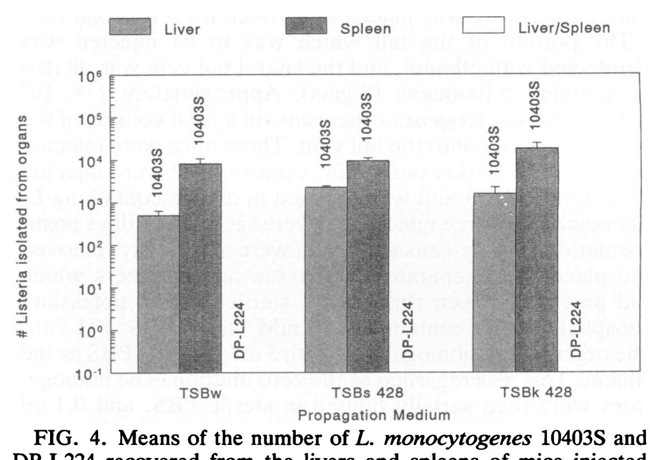

FIG. 4. Means of the number of *L. monocytogenes* 10403S and DP-L224 recovered from the livers and spleens of mice injected intravenously with approximately 2×10^4 viable cells grown in either TSBw, TSBs 428, or TSBk 428. Error bars represent the standard errors of the means. ▨, liver; ▩, spleen, □, liver/spleen.

TABLE 1. ALD_{50}s of *L. monocytogenes* 10403S inoculated i.g. or i.p. into mice after growth in media containing no salt, 428 mM NaCl, or 428 mM KCl

Condition	i.g. CFU		i.p. CFU	
	ALD_{50}	95% Confidence interval	ALD_{50}	95% Confidence interval
TSBw	1.4×10^{7a}	5.8×10^5–3.4×10^8	3.1×10^{4b}	1.8×10^4–5.4×10^4
TSBs 428	8.5×10^{7a}	7.4×10^6–9.8×10^8	1.4×10^{4b}	9.0×10^2–2.0×10^5
TSBk 428	5.1×10^{7a}	5.4×10^6–4.8×10^8	2.3×10^{4b}	1.3×10^4–4.1×10^4

[a] Values sharing the same superscript letter are not significantly different.

the virulence of *L. monocytogenes* 10403S grown under the various conditions was found.

ALD_{50}s. The ALD_{50}s are given in Table 1. The ALD_{50}s were not significantly different ($P < 0.05$) between any of the growth conditions after either i.g. or i.p. infection. Strain 10403S gave an i.g. ALD_{50} that was approximately 1,000 times higher than the i.p. ALD_{50}. An ALD_{50} could not be calculated for the nonhemolytic strain DP-L224 (data not shown).

DISCUSSION

The two listerial strains examined in the study described here exhibited similar results with regard to their CA and SOD activities when compared with the results found by Dallmier and Martin (9). Strains 10403S and DP-L224 displayed both stimulated and depressed CA and SOD activities (except for strain 10403S in TSBk 428) when grown in media with identical formulations. This suggests that alteration of the hemolysin gene to obtain the ahemolytic mutant DP-L224 does not significantly alter the production of CA or SOD. In the comparison of NaCl and KCl, the stimulation of CA activity was probably not due to the cation.

When cells of SOD-producing bacteria are exposed to increased levels of O_2, induction of manganese-containing SOD and, to a lesser degree, CA occurred (25). Welch (30) and Welch et al. (31) also noted that increased aeration increased CA and SOD production. Dallmier and Martin (9) have suggested that O_2 levels in *L. monocytogenes* may be stimulated by intermediate salt levels, which may increase SOD activity. The results found for *L. monocytogenes* 10403S and DP-L224 when propagated in NaCl- or KCl-containing medium are in agreement, since both strains exhibited enhanced CA and SOD activities.

Hemolysin production has been shown to be stimulated under various conditions. Dallmier and Martin (9) showed that LLO activity is stimulated in growth medium containing 428 mM NaCl. Reduced aeration has also been shown to increase hemolysin activity (2), while maintenance of cultures at reduced temperatures for extended periods of time enhanced the hemolytic activity (10, 12, 13). Hemolytic activity was strongly enhanced when *L. monocytogenes* was propagated in charcoal-treated broth as compared with that when it was propagated in untreated broth (11). In addition, the production of exonuclease by *Staphylococcus aureus* was increased four- to fivefold when it was cultured in media containing 171 to 513 mM NaCl, respectively (29). The results for the LLO activity by *L. monocytogenes* 10403S compared favorably with the findings for staphylococcal exonuclease and with those found by Dallmier and Martin (9). Since LLO is an exoenzyme, the intermediate NaCl or KCl concentration may allow for more efficient LLO transport across the membrane or the permeation of an inducer into the cell, or a global type of regulation may occur when the cells are stressed.

The two *L. monocytogenes* strains were propagated in TSBw, TSBs 428, and TSBk 428 and were injected intravenously in equal numbers into mice. The spleens and livers were then removed to measure the severity of infection (number of organisms present in these organs). No significant difference between cells grown under any of the various conditions was found. The results indicate that stimulation of virulence factor activities, specifically LLO, did not enhance the virulence of strain 10403S or DP-L224. These results agree with those of other investigators. Maintenance at 4°C on solid medium for extended periods or brief incubations of overnight cultures at 56°C made *L. monocytogenes* SLCC 1444 hyperhemolytic (13). These hyperhemolytic bacteria were injected into mice, and no increase in virulence was seen when compared with that of bacteria with normal hemolytic status. They concluded that LLO is required for virulence but that the levels of LLO are not directly proportional to virulence.

The ALD_{50}s after i.g. or i.p. infection were not significantly influenced by the growth conditions examined. Pine et al. (22) found similar results for *L. monocytogenes* grown in milk and for *L. monocytogenes* cells cultured on tryptose blood agar. They noted that growth in a selective environment did not select for a more virulent population of organisms. The ALD_{50}s after i.g. and i.p. infection were approximately 10^7 and 10^4 CFU, respectively. These values were similar to those obtained by other researchers, who suggested that i.g. ALD_{50}s would be 100 to 1,000 times greater than i.p. ALD_{50}s (1, 19).

The results from the present study (enhanced CA and SOD activities) appear to support the findings of Welch (30). Welch (30) determined the ALD_{50}s and the CA and SOD activities of five strains of *L. monocytogenes*. Welch was unable to correlate virulence with SOD activity. However, virulent strains were shown to possess relatively higher CA and SOD activities. The levels of CA and SOD produced appeared to vary between strains. Dallmier and Martin (8) noted that strains of *L. monocytogenes* with high CA activities also possessed the highest SOD activities. Two of the strains examined by Welch (30) had CA and SOD activities of 167 and 334 and 45 and 51 U/mg of protein, respectively. The corresponding ALD_{50}s were 5×10^3 and 5×10^4 CFU. The CA and SOD activities of strain 10403S grown in TSBw at 37°C were 20 and 93 U/mg of protein, respectively. The CA activity was less than that noted by Welch (30), while the SOD activity was approximately 50% greater. However, the i.p. ALD_{50} for strain 10403S was 3.1×10^4 CFU, which was comparable to that found by Welch (30).

The results presented here indicate that propagation of *L. monocytogenes* with various salt concentrations at 37°C causes increased production of CA, SOD, and LLO. However, in agreement with the results of Welch (30) and Katharious et al. (13), high levels or increased levels of production of these enzymes could not be attributed to enhanced virulence in strain 10403S. The evidence suggests that CA and SOD do not confer on, but may contribute to, the pathogenicity of *L. monocytogenes*. Since CA and SOD both act to detoxify oxygen by-products of their own metabolism and from phagocytic cells, strains with reduced activities of either enzyme may still be able to defend themselves against toxic oxygen species. In fact, a CA-negative strain has been shown to retain its virulence (16). These results suggest that in *L. monocytogenes* 10403S, growth in foods containing NaCl and KCl may not influence the virulence of *L. monocytogenes*. However, these results do not exclude the possible role of other environmental factors (temperature) on the expression of other virulence genes (4, 14, 17, 23).

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REFERENCES

1. **Audurier, A., P. Pardon, J. Marly, and F. Lantier.** 1980. Experimental infection of mice with *Listeria monocytogenes* and *L. innocua*. Ann. Microbiol. (Paris) **131B:**47–57.
2. **Benedict, R. C.** 1990. *Listeria monocytogenes*: physiology and metabolism, p. 13–24. *In* A. J. Miller, J. L. Smith, and G. A. Somkuti (ed.), Foodborne listeriosis. Elsevier, New York.
3. **Chakraborty, T., and W. Goebel.** 1988. Recent developments in the study of virulence in *Listeria monocytogenes*. Curr. Top. Microbiol. Immunol. **138:**41–58.
4. **Chakraborty, T., M. Leimeister-Wächter, E. Domann, M. Hartl, W. Goebel, T. Nichterlein, and S. Notermans.** 1992. Coordinated regulation of virulence genes in *Listeria monocytogenes* requires the product of the *prfA* gene. J. Bacteriol. **174:**568–574.
5. **Cossart, P., M. F. Vicente, J. Mengaud, F. Baquero, J. C. Peres-Diaz, and P. Berche.** 1989. Listeriolysin O is essential for virulence of *Listeria monocytogenes*: direct evidence obtained by gene complementation. Infect. Immun. **57:**3629–3636.
6. **Cowart, R. E., and B. G. Foster.** 1985. Differential effects of iron on the growth of *Listeria monocytogenes*: minimum requirements and mechanisms of acquisition. J. Infect. Dis. **151:**721–730.
7. **Czuprynski, C. J., J. F. Brown, and J. T. Roll.** 1989. Growth at reduced temperatures increases the virulence of *Listeria monocytogenes* for intravenously but not intragastrically inoculated mice. Microb. Pathog. **7:**213–223.
8. **Dallmier, A. W., and S. E. Martin.** 1988. Catalase and superoxide dismutase activities after heat injury of *Listeria monocytogenes*. Appl. Environ. Microbiol. **54:**581–582.
9. **Dallmier, A. W., and S. E. Martin.** 1990. Catalase, superoxide dismutase, and hemolysin activities and heat susceptibility of *Listeria monocytogenes* after growth in media containing sodium chloride. Appl. Environ. Microbiol. **56:**2807–2810.
10. **Durst, J.** 1975. The role of temperature factors in the epidemiology of listeriosis. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **233:**72–74.
11. **Geoffroy, C., J. L. Gaillard, J. Alouf, and P. Berche.** 1989. Production of thiol-dependent hemolysins by *Listeria monocytogenes* and related species. J. Gen. Microbiol. **135:**481–487.
12. **Girard, K. F., A. J. Sbarra, and W. A. Bardawil.** 1963. Serology of *Listeria monocytogenes*. I. Characteristics of the soluble hemolysin. J. Bacteriol. **85:**349–355.
13. **Kathariou, S., J. Rocourt, H. Hof, and W. Goebel.** 1988. Levels of *Listeria monocytogenes* hemolysin are not directly proportional to virulence in experimental infections of mice. Infect. Immun. **56:**534–536.
14. **Kuhn, M., S. Kathariou, and W. Goebel.** 1988. Hemolysin supports survival but not entry of the intracellular bacterium *Listeria monocytogenes*. Infect. Immun. **57:**79–82.
15. **Lamont, R. J., R. Postlethwaite, and A. P. MacGowan.** 1988. *Listeria monocytogenes* and its role in human infection. J. Infect. **17:**7–28.
16. **Leblond-Francillard, M., J. L. Gaillard, and P. Berche.** 1989. Loss of catalase activity in Tn*1545*-induced mutants does not reduce growth of *Listeria monocytogenes* in vivo. Infect. Immun. **57:**2569–2573.
17. **Leimeister-Wächter, M., E. Domann, and T. Chakraborty.** 1992. The expression of virulence genes in *Listeria monocytogenes* is thermoregulated. J. Bacteriol. **174:**947–952.
18. **Lovett, J.** 1989. *Listeria monocytogenes*, p. 283–310. *In* M. Doyle (ed.), Foodborne bacterial pathogens. Marcel Dekker, Inc., New York.
19. **MacDonald, T. T., and P. B. Carter.** 1980. Cell-mediated immunity to intestinal infection. Infect. Immun. **28:**516–523.
20. **McClure, P. J., T. A. Roberts, and P. Otto Oguru.** 1989. Comparison of the effects of sodium chloride, pH and temperature on the growth of *Listeria monocytogenes* on gradient plates and in liquid medium. Lett. Appl. Microbiol. **9:**95–99.
21. **Mengaud, J., J. Chenevert, C. Geoffroy, J.-L. Gaillard, and P. Cossart.** 1987. Identification of the structural gene encoding the SH-activated hemolysin of *Listeria monocytogenes*: listeriolysin O is homologous to streptolysin O and pneumolysin. Infect. Immun. **55:**3225–3227.
22. **Pine, L., G. B. Malcolm, and B. D. Plikaytis.** 1990. *Listeria monocytogenes* intragastric and intraperitoneal approximate 50% lethal doses for mice are comparable, but death occurs earlier by intragastric feeding. Infect. Immun. **58:**2940–2945.
23. **Portnoy, D. A., T. Chakraborty, W. Goebel, and P. Cossart.** 1992. Molecular determinants of *Listeria monocytogenes* pathogenesis. Infect. Immun. **60:**1263–1267.
24. **Portnoy, D. A., P. S. Jacks, and D. J. Hinrichs.** 1988. Role of hemolysin for the intracellular growth of *Listeria monocytogenes*. J. Exp. Med. **167:**1459–1471.
25. **Schiavone, J. R., and H. M. Hassan.** 1988. The role of redox in the regulation of maganese-containing superoxide dismutase biosynthesis in *Escherichia coli*. J. Biol. Chem. **263:**4269–4273.
26. **Seeliger, H. P. R., and H. J. Welshimer.** 1974. Genus *Listeria*, p. 593–596. *In* R. E. Buchanan and N. E. Gibbons (ed.), Bergey's manual of determinative bacteriology, 7th ed. The Williams & Wilkins Co., Baltimore.
27. **Shahamat, M., A. Seaman, and M. Woodbine.** 1980. Survival of *Listeria monocytogenes* in high salt concentrations. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **246:**506–511.

27a. **Sigma Chemical Co.** 1990. Technical Bulletin no. 210. Sigma Chemical Co., St. Louis.

28. **Trevors, J. T.** 1986. A BASIC program for estimating LD_{50} values using the IBM-PC. Bull. Environ. Contam. Toxicol. **37:**18–26.
29. **Udou, T., and Y. Ichikawa.** 1980. Effect of sodium chloride on the activity and production of staphylococcal exonuclease. J. Gen. Microbiol. **116:**69–74.
30. **Welch, D. F.** 1987. Role of catalase and superoxide dismutase in the virulence of *Listeria monocytogenes*. Ann. Inst. Pasteur. Microbiol. **138:**265–268.
31. **Welch, D. F., C. P. Sword, S. Brehm, and D. Dusanic.** 1979. Relationship between superoxide dismutase and pathogenic mechanisms of *Listeria monocytogenes*. Infect. Immun. **23:**863–872.